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13. ABSTRACT (Maximum 200 Words)

Tumor progression induces the growth of endothelial cells by releasing angiogenic factors. This is accompanied by down-regulation of local tissue inhibitors of endothelian cell proliferation such as angiostatin and endostatin. Both proteins target normal endothelial cells and effectively regress large tumors in animals. However, animal studies demonstrate that an effective treatment requires long-term administration of angiogenesis inhibitors. Thus, delivery of angiogenesis inhibitor genes to tumor sites should increase local concentration of these proteins, leading to the retardation of tumor progression and metastasis. During the past fiscal year, we have established stable human prostate cancer cell lines derived from PC3 cells transduced with HIV7/endo, HIV7/angio or both together. Expression of the angiogenesis inhibitors did not affect the proliferation of PC3 cells significantly. These PC cell lines produced and secreted sufficient amounts of angiogenesis inhibitors to inhibit the proliferation of primary human primary human umbilical vein endothelial cells (HUVEC) in culture. We have subcutaneous implanted these cell lines onto nude mice and will evaluate the effect of angiogenesis inhibitors on tumor growth *in vivo*.

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Introduction

Tumor progression induces the growth of endothelial cells by releasing angiogenic factors. This is accompanied by down-regulation of local tissue inhibitors of endothelian cell proliferation such as angiostatin and endostatin. Both proteins target normal endothelial cells and effectively regress large tumors in animals. However, animal studies demonstrate that an effective treatment requires long-term administration of angiogenesis inhibitors. Thus, delivery of angiogenesis inhibitor genes to tumor sites should increase local concentration of these proteins, leading to the retardation of tumor progression and metastasis. We propose to use HIV vectors to deliver the endostatin and angiostatin genes into human prostate cancer cell lines in culture. The effect of these two proteins will be evaluated by tumor formation and metastasis in nude mice grafted with the transduced cells. During the past fiscal year, we have established stable human prostate PC3 cell lines expressing either endostatin, angiostatin or both angiogenesis inhibitors together. The expression of these proteins had no effect on the growth rate of PC3 cells. However, culture supernatant harvested from these cell lines exhibited growth inhibition of primary human endothelial cells in culture. We used two different assays to detect potential contamination of replication competent lentivirus (RCL) in these established cell lines and failed to detect the presence RCL. We have now implanted these cells onto nude mice and are in the process of evaluating the effect of the angiogenesis inhibitor on tumor growth *in vivo*.

Body

During this fiscal year, we have established stable cell clones derived from human prostate cancer cell line, PC3, expressing angiogenesis inhibitors. These transduced cells will then be tested for their tumorigenicity *in vivo*. PC3 cells were transduced with either HIV7/GFP containing only the GFP gene, HIV7/endo containing the endostatin cDNA, HIV7/angio containing the angiostatin cDNA or both HIV7/endo and HIV7/angio at high multiplicity of infection (MOI). Since both HIV7/endo and HIV7/angio also carry the GFP gene, the efficiency of PC3 transduction can be determined by the FACS analysis of GFP+ cells. As shown in Fig. 1, at an MOI of 5, approximately 20% of the PC3 cells became GFP+. In contrast, more than 90% of HIV7/GFP transduced cells became GFP+ with the same MOI. To increase the fraction of cells expressing the angiogenesis inhibitor, PC3 cells were repeatedly transduced with the HIV vectors at the MOI of 5. As shown in Table 1, the fraction of GFP+ cells increased to 84% and 95% when transduced with HIV7/endo and HIV7/angio, respectively. To determine the combined effect of endostatin and angiostatin, we mixed equivalent fractions of HIV7/endo and HIV7/angio transduced PC3 cells. The GFP+ fraction in this population was determined to be 80% (HIV7/endo + HIV7/angio in Table 1). Since endostatin was tagged with the influenza HA peptide [1], to determine whether angiostatin was expressed and at what level, the culture supernatant or cell extract from PC3 cells transduced with either HIV7/GFP or

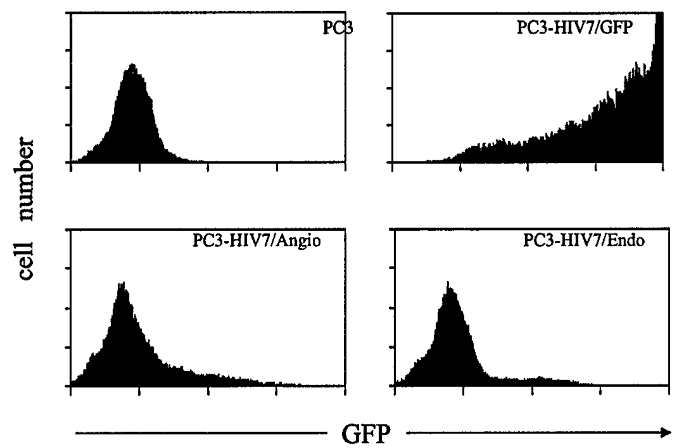

Fig. 1. FACS analysis of HIV vector transduced PC3 cells.

Table 1. Percentage of GFP+ PC3 cells transduced with different HIV vectors.

mock	HIV7/ GFP	HIV7/ endo	HIV7/ angio	HIV7/endo + HIV7/angio
0%	95%	84%	95%	80%

HIV7/angio were prepared and subjected to Western blot analysis using a HA-specific antibody. As shown in Fig. 2, angiostatin expression was readily detectable in the culture supernatant and the cell extract derived from PC3 cells transduced with HIV7/angio but not in PC3 cells transduced with HIV7/GFP. Since the antibody specific for endostain is not currently available, we were not able to perform a similar experiment to evaluate endostatin expression. To determine whether expression of the angiogenesis inhibitor affects the proliferation of PC3 cells, the growth rate of these cell lines were determined. As shown in Fig. 3, no distinct difference in the growth rate of these cells could be detected. To determine whether the angiogenesis inhibitors produced from these cell lines were capable of blocking the proliferation of normal endothelial cells, the culture supernatant from each cell lines was harvested and concentrated with Centricon [2].

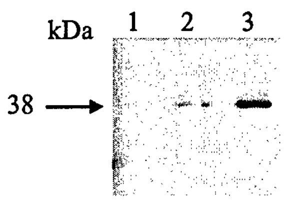

Fig. 2. Western blot analysis of angiostatin expression in HIV7/angio transduced PC3 cells. Lanes 1& 2 contain concentrated culture supernatants from PC3 cells transduced with HIV7/GFP and HIV7/angio, respectively; lane 3 contains the cell extract from PC3 cells transduced with HIV7/angio.

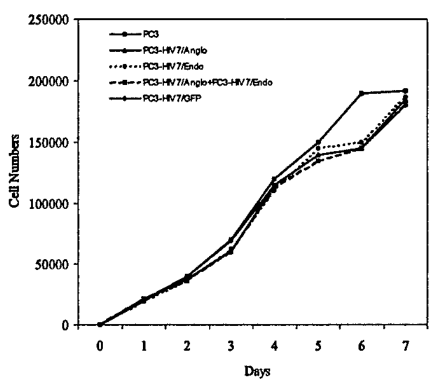

Fig. 3. The growth rate of HIV vector-transduced PC3 cells.

The concentrated supernatant was applied to primary human umbilical vein endothelial cells (HUVEC) and cell proliferation was monitored by the MTT assay. We have shown in the last progress report that direct transduction of HUVECs with HIV7/endo or HIV7/angio led to a decrease in the cell proliferation rate in culture. As shown in Fig. 4, the culture supernatants harvested from PC3 cells transduced with HIV7/endo, HIV7/angio and HIV7/endo plus HIV7/angio inhibited HUVEC proliferation whereas the supernatants harvested from PC3 cells transduced with HIV7/GFP had little effect on HUVEC proliferation. A combination of endostatin and angiostatin, however, did not have any additive effect on the inhibition of HUVEC proliferation. This experimental approach mimics the *in vivo* gene therapy conditions where vector-transduced cancer cells express and secrete the angiogenesis inhibitor to block normal endothelial cell proliferation. Before implanted the transduced cells into nude mice to assess the effect of angiogenesis inhibitors on tumor growth *in vivo*, we used two assays to detect the potential contamination of replication competent lentivirus (RCL) in the established PC3 lines. In the first assay, the transduced PC3 cells were cultured continuously for over a month and the culture supernatant was collected during this period and the p24 level was then determined using a commercially available Elisa kit. As shown in Fig. 5, no significant level of p24 could be detected in any of the transduced PC3 cells at the end of this culture period. The values of p24 fell below the detection limit of 7 pg/ml. In the second assay, genomic DNA from the transduced cells was prepared at the end of the culture period and subjected to DNA PCR analysis to detect potential RCL. Two pairs of PCR primers were used: pol-1/pol-2 amplify a 386-bp fragment in the reverse transcriptase (RT) gene. RCL is expected to contain this gene in order to replicate and spread. As a control, vec-1/vec-2 amplify a 270-bp fragment presence in the 5' untranslated region of HIV-1. Both RCL and the HIV

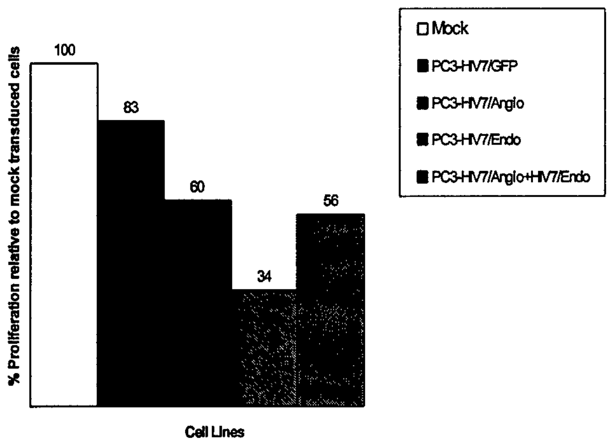

Fig. 4. Inhibition of HUVEC proliferation by the culture supernatant of HIV vector-transduced PC3 cells.

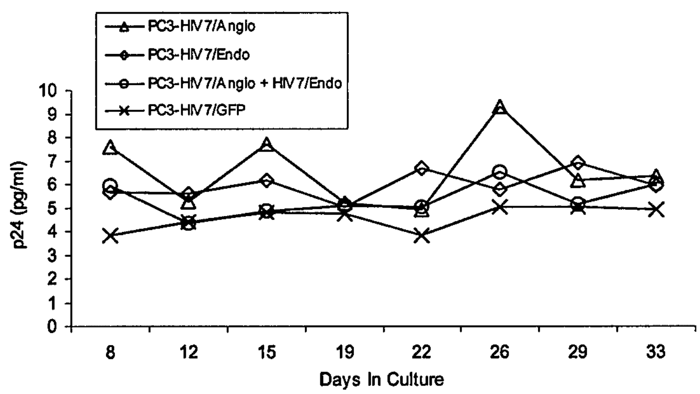

Fig. 5. The level of p24 in the supernatant of HIV vector-transduced PC3 cells.

vectors used in this study are expected to contain this sequence. As shown in Fig. 6, the 386-bp fragment could only be detected from PCR amplification of pCMV-HIV-1 which contains the RT gene (lanes 6, 7, 18 &19). None of DNA from the transduced cells contained this gene. In contrast, the 270-bp fragment was readily detectable in the DNA samples isolated from the transduced cells. We concluded that the PC3 cell lines established were free of RCL contamination. To assay the effect of angiogenesis inhibitor expression *in vivo*, we first determined that approximately 2 x 10^6 PC3 cells were required to induce tumor in nude mice within 10 days with subcutaneous implantation. Five groups of nude mice with five mice in each group were then implanted with 2 x 10^6 PC3, PC3/HIV7/GFP, PC3/HIV7/endo, PC3/HIV7/angio and PC3/HIV7/endo plus HIV7/angio, respectively. This is ongoing and tumor growth *in vivo* will be followed by measuring the tumor size.

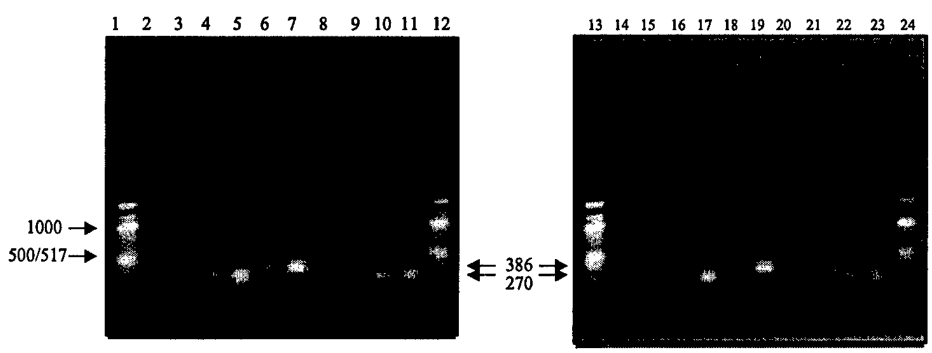

Fig. 6. PCR analysis of the genomic DNA from HIV vector-transduced PC3 cells. Lanes 1, 12, 13 & 24: 100-bp DNA ladders. Lanes 2-5: HIV7/angio transduced cells. Lanes 2 & 3: 10 ng and 100 ng DNA amplified by pol-1/pol-2. Lanes 4 &5: 10 ng and 100 ng DNA amplified by vec-1/vec-2. Lanes 6 & 7: 0.1 ng and 1 ng pCMV-HIV-1 amplified by pol-1/pol-2. Lanes 18 &19 are identical to lanes 6 & 7. Lanes 8-11: similar to lanes 2-5 except that the DNA was derived from HIV7/endo transduced cells. Lanes 14-17: similar to lanes 2-5 except that the DNA was derived from HIV7/angio + HIV7/endo transduced cells. Lanes 20-23: similar to lanes 2-5 except that the DNA was derived from HIV7/CMV-GFP transduced cells.

Key Research Accomplishments

1. Establish stable PC3 cell lines stably expressing endostatin, angiostatin, or both.

2. Demonstrate the inhibition of primary endothelial cell proliferation with the angiogenesis inhibitors produced from the stable PC3 cell lines

3. Demonstrate the absence of RCL in the established cell lines and proceed with nude mice implantation of these cell lines to evaluate tumor formation *in vivo*

Reportable Outcomes

1. Establish PC3 cell lines stably expressing endostatin and angiostatin. These cell lines can be used to evaluate prostate tumor progression *in vivo*.

2. Claudia Kowolik, a Postdoctoral Fellow, received training in HIV vector production, RCL detection and tumor cell implantation onto nude mice.

Conclusion

1. Stable human PC3 prostate cancer cell lines expressing endostatin and angiostatin were established.

2. Expression of these angiogenesis inhibitors had no effect on PC3 cell proliferation in culture.

3. The angiogenesis inhibitors produced from PC3 cells, however, inhibited the proliferation of primary human endothelial cells in culture.

4. No RCL could be detected from these stable PC cell lines. These cell lines therefore can be used to implant onto nude mice.

References

1. Tanaka T, Cao Y, Folkman J, Fine HA. **Viral vector-targeted antiangiogenic gene therapy utilizing an angiostatin complementary DNA.** *Cancer Res.* 1998,58:3362-3369.

2. Nguyen JT, Wu P, Clouse ME, Hlatky L, Terwilliger EF. **Adeno-associated virus-mediated delivery of antiangiogenic factors as an antitumor strategy.** *Cancer Res.* 1998,58:5673-5677.

Appendices

1. Curriculum Vitae

CURRICULUM VITAE

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Education:

1972 - 1976	B.S. in Biology, Fu Jen Catholic University, Taipei, Taiwan
1977 - 1979	M.S. in Molecular Biology, University of Texas at Dallas, Dallas, Texas
1980 - 1983	Ph.D. in Molecular Biology, University of Texas at Dallas, Dallas, Texas

Postdoctoral Training:

1983 - 1986	Postdoctoral Fellow, Department of Pediatrics, University of California at San Diego, La Jolla, California
1986 - 1987	Postgraduate Biochemist, Department of Pediatrics, University of California at San Diego, La Jolla, California

Professional Appointment:

1987 - 1994	Assistant Adjunct Professor, Department of Pediatrics, University of California at San Diego, La Jolla, California
1994 - 1996	Assistant Professor, Department of Pediatrics, City of Hope National Medical Center, Duarte, California
1997 – 1998	Senior Research Scientist, Chiron Technologies, Center for Gene Therapy, San Diego, California
1998 – present	Associate Professor, Department of Virology, City of Hope National Medical Center, Duarte, California

Awards:

1977 - 1983	Research and Teaching Assistantships, University of Texas at Dallas
1983 - 1986	Postdoctoral Fellowship, University of California at San Diego

Teaching Experience:

1976 - 1977	Teaching Assistant for laboratory courses in Biochemistry and Microbiology, Department of Biology, Fu Jen Catholic University, Taipei, Taiwan
1979 - 1983	Teaching Assistant for the laboratory course in Microbiology, Department of Molecular Biology, University of Texas at Dallas, Dallas, Texas
1994 - 1996	Instructor for the course of Molecular and Cellular Biology in the Graduate Program, City of Hope, Duarte, California
2001-present	Instructor for Breast Cancer Biology in the Graduate Program, City of Hope, Duarte, California

Scientific Committees:

Ad hoc Reviewer for NIDDK study section (1991)

Ad hoc Reviewer for Wellcome Trust Foundation (1991)

Ad hoc Reviewer for NIDDK study section (1992)

Ad hoc Reviewer for NIDDK study section (1994)

Reviewer for NIH SBIR Special Study Section (2000- 2001)

Ad hoc Reviewer for NIH Recombinant DNA Advisory Committee (2001)

Grant Support:

Project Title: Hematopoietic cell transplantation for hematologic malignancy

P01 CA30206	NIH/NCI	4/1/00 - 3/31/05
Role: Co-PI (PI: S. Forman)	20 % effort	

Project Title: Transduction of ribozymes into CD34+ stem cell in AIDS

P01 A146030 NIH 9/1/99-8/31/03
Role: Project Leader (PI: J. Zaia) 30% effort

Project Title: Anti-angiogenic gene therapy of prostate cancer with lentiviral vectors
New Investigator Award Prostate Cancer Research Program, US Army
03/01/01-02/28/04
Role: PI 10% effort

Patent granted

Patent No. 6,133,027
Title: Inducible expression system
Inventors: **Yee; Jiing-Kuan**; Friedmann; Theodore; Chen; Shin-Tai
Assignee: City of Hope (Duarte, CA); The Regents of the University of California (Oakland, CA)

Patent No. 5,817,491
Title: VSV G pseudotyped retroviral vectors
Inventors: **Yee; Jiing-Kuan**; Emi; Nobuhiko; Friedmann; Theodore; Jolly; Douglas J.; Barber; Jack R.
Assignee: The Regents of the University of California (Oakland, CA); Chiron Viagene, Inc. (Emeryville, CA)

Patent No. 5,739,018
Title: Packaging cell lines for pseudotyped retroviral vectors
Inventors: Miyanohara; Atsushi; **Yee; Jiing-Kuan**; Chen; Shin-Tai; Prussak; Charles Edward; Friedmann; Theodore
Assignee: The Regents of the University of California (Oakland, CA); City of Hope (Duarte, CA)

Patent No. 5,670,354
Title: Use of VSV-G pseudotyped vectors for transfer of genes into embryos
Inventors: Burns; Jane C.; **Yee; Jiing-Kuan**; Friedmann; Theodore
Assignee: The Regents of the University of California (Oakland, CA)

Patent No. 5,512,421
Title: Generation, concentration and efficient transfer of VSV-G pseudotyped retroviral vectors
Inventors: Burns; Jane C.; **Yee; Jiing-Kuan**; Friedmann; Theodore
Assignee: The Regents of the University of California (Oakland, CA)

Publications:

1. **Yee, J.-K.** and Marsh, R.C. "Alignment of a Restriction Map with the Genetic Map of Bacteriophage T4." **Journal of Virology 38**, 115-124 (1981).

2. **Yee, J.-K.** and Marsh, R.C. "Locations of Bacteriophage T4 Origins of Replication." **Journal of Virology 54**, 271-277 (1985).

3. Gruber, H.E., Finley, K.D., Hershberg, R.M., Katzman, S.S., Laikind, P.K., Seegmiller, J.E., Friedmann, T., **Yee, J.-K.** and Jolly, D.J. "Retroviral Vector-Mediated Gene Transfer into Human Hematopoietic Progenitor Cells." **Science 230**, 1057-1061 (1985).

4. Gruber, H.E., Finley, K.D., Lutchman, L.A., Hershberg, R.M., Scott, S.K., Laikind, P.K., Meyers, E.N., Seegmiller, J.E., Friedmann, T., **Yee, J.-K.**, and Jolly, D.J., "Insertion of Hypoxanthine Phosphoribosyltransferase cDNA into Human Bone Marrow Cells by a Retrovirus," in **Purine and Pyrimidine Metabolism in Man V**, Nyhan, W.L., Thompson, L.F. and Watts, R.W.E., eds., Plenum Publishing Corporation, pp.171-175 (1986).

5. **Yee, J.-K.**, Jolly, D.J., Moores, J.C., Respess, J.G. and Friedmann, T. "Gene Expression from a Transcriptionally Disabled Retroviral Vector Containing an Internal Promoter," **Cold Spring Harbor Symposia on Quantitative Biology**, pp.1021-1026 (1986).

6. Gruber, H.E., Finley, K.D., Luchtman, L.A., Hershberg, R.M., Katzman, S.S., Laikind, P.K., Meyers, E.N., Seegmiller, J.E., Friedmann, T. and **Yee, J.-K.** "Insertion of hypoxanthine phosphoribosyltransferase cDNA into human bone marrow cells by a retrovirus." **Adv. Exp. Med. Biol. 195**, 171-175 (1986).

7. **Yee, J.-K.**, Jolly, D.J., Miller, A.D., Willis, R.C. and Friedmann, T. "Epitope Insertion into the Human Hypoxanthine Phosphoribosyltransferase Protein and Detection of the Mutant Protein by an Anti-Peptide Antibody." **Gene 53**, 97-104 (1987).

8. Jolly, D.J., **Yee, J.-K.** and Friedmann, T. "Retroviral Vector Design for HPRT Gene Transfer," in **Drug and Enzyme Targeting, Methods in Enzymology,Vol.149**, Green,R. and Widder, K.J., eds., Academic Press, Inc., Orlando, Fla., pp.10-25 (1987).

9. Wolff, J.A., **Yee, J.-K.,** Skelly, H., Moores, J.C., Respess, J.G., Friedmann, T. and Leffert, H. "Expression of Retrovirally Transduced Genes in Primary Cultures of Adult Rat Hepatocytes." **Proc. Natl. Acad. Sci .USA 84**, 3344-3348 (1987).

10. **Yee, J.-K.,** Moores, J.C., Jolly, D.J., Wolff, J.A., Respess, J.G. and Friedmann, T. "Gene Expression from Transcriptionally Disabled Retroviral Vectors." **Proc. Natl. Acad. Sci. USA 84**, 5197-5201 (1987).

11. Wolff, J.A., Moores, J.C., **Yee, J.K.**, Respess, J.G., Skelly, H., Leffert, H. and Friedmann, T. "Retroviral-Mediated Transduction into Hepatocytes in vitro." **Proc.19th Miami Winter Symposium 7**, 144-145 (1987).

12. Huang, H.-J.S., **Yee, J.-K.**, Shew, J.-Y., Chen, P.-L., Bookstein, R., Friedmann, T., Lee, E.Y.-H.P. and Lee, W.-H. "Suppression of the Neoplastic Phenotype by Replacement of the Human Retinoblastoma Gene Product in Retinoblastoma and Osteosarcoma Cells." **Science 242**, 1553-1556 (1988).

13. Gage, F., Wolff, J.A., Rosenberg, M., Xu, L., **Yee, J.-K.,** Shultz, C. and Friedmann, T. "Grafting Genetically Modified Cells to the Brain." **Neuroscience 23**, 795-807 (1988).

14. Gage, F.H., Wolff, J.A., Rosenberg, M.B., Li Xu, Yee, **J.-K.,** Shults, C. and Friedmann, T. "Implantation of genetically engineered cells to the brain", in **Progress in Brain Res. 78**, 651-658 (1988).

15. Li, X., **Yee, J-K.,** Wolff, J.A. and Friedmann, T. "Factors affecting long-term stability of Moloney murine leukemia virus-based vectors." **Virology 171**, 331-341 (1989).

16. **Yee, J.-K.** "A liver-specific enhancer in the core promoter region of human Hepatitis B virus." **Science, 246,** 658-661 (1989).

17. Friedmann, T., Li, X., Wolff, J., **Yee, J.-K.** and Miyanohara, A. "Retrovirus vector-mediated gene transfer into hepatocytes." **Mol. Biol. Med. 6**, 117-125 (1989).

18. Emi, N., Friedmann, T. and **Yee, J.-K.** "Pseudotype formation of murine leukemia virus with the G protein of vesicular stomatitis virus." **J. Virol. 65**, 1202-1207 (1991).

19. Levine, F., **Yee, J.-K.** and Friedmann, T. "Efficient gene expression from a dicistronic transcriptional unit in an improved retroviral vector." **Gene 108**, 167-174 (1991).

20. Cheng, J., **Yee, J.-K.**, Yeargin, J., Friedmann, T. and Haas, M. "Suppression of acute lymphoblastic leukemia by the human wild-type p53 gene." **Cancer Research 52,** 222-226 (1992).

21. Su, H. and **Yee, J.-K.** "Regulation of hepatitis B virus gene expression by its two enhancers." **Proc. Natl. Acad. Sci. USA 89,** 2708-2712 (1992).

22. Chen, S.-T., Su, H. and **Yee, J.-K**. "Adenovirus E1A-mediated transcriptional repression of hepatitis B virus enhancer activity." **J. Virol. 66,** 7452-7460 (1992).

23. Chen,S.-T., La Porte, P. and **Yee, J.-K.** "Mutational analysis of hepatitis B virus enhancer 2." **Virology 196,** 652-659 (1993).

24. Burns, J.C., Friedmann, T., Driever, W., Burrascano, M. and **Yee, J.K.** "VSV-G pseudotyped retroviral vectors: concentration to very high titer and efficient gene transfer into mammalian and nonmammalian cells." **Proc. Natl. Acad. Sci. USA 90,** 8033-8037 (1993).

25. Abe, A., Takeo, T., Emi, N., Tanimoto, M., Ueda, R., **Yee, J.-K.**, Friedmann, T. and Saito, H. "Transduction of a drug-sensitive toxic gene into human leukemia cell lines with a novel retroviral vector." **Proc. Soc. Exp. Biol. Med. 203,** 354-359 (1993).

26. Yamada, O., Yu, M., **Yee, J.K.**, Kraus,G., Looney, D.and Wong-Staal, F. "Intracellular immunization of human T cells with a hairpin ribozyme against human immunodeficiency virus type 1." **Gene Ther.** 1, 38-45 (1994).

27. **Yee, J.-K.**, Friedmann, T. and Burns, J.C. "Generation of high-titer pseudotyped retroviral vectors with very broad host range." **Methods in Cell Biology**, 43, 99-112 (1994).

28. Burns, J.C., Matsubara, T., Lozinski, G., **Yee, J.-K.**, Washabaugh, C.H. and Tsonis, P.A. "Pantropic retroviral vector-mediated gene transfer, integration, and expression in newt limb blastema cells." **Dev. Biol.** 165, 285-289 (1994).

29. Lin, S., Gaiano, N., Culp, P., Burns, J.C. Friedmann, T., **Yee, J.K.** and Hopkins, N. "Integration and germ line transmission of a pseudotyped retroviral vector in zebrafish." **Science** 265, 666-669 (1994).

30. **Yee, J.-K.**, Miyanohara, M., LaPorte, P., Bouic, K., Burns, J.C. and Friedmann, T. "Generation of high titer, pantropic retroviral vectors: Efficient gene transfer into hepatocytes." **Porc. Natl. Acad. Sci. USA** 91, 9564-9568 (1994).

31. Runnebaum, I.B., **Yee, J.K.,** Kieback, D.G., Sukumar, S. and Friedmann, T. "Wild-type p53 suppresses the malignant phenotype in breast cancer cells containing mutant p53 alleles." **Anticancer Res.** 14, 1137-1144 (1994).

32. Miyanohara, A., **Yee, J.K.,** Bouic, K., LaPorte, P. and Friedmann, T. "Efficient in vivo transduction of the neonatal mouse liver with pseudotyped retroviral vectors." **Gene Ther.** 2, 138-142 (1995).

33. Yu,M., Poeschla,E., Yamada,O., Degrandis,P., Leavitt,M.C., Heusch,M., **Yee,J.K.**, Wong-Staal,F. and Hampel.A. "In vitro and in vivo characterization of a second functional hairpin ribozyme against HIV-1." **Virology** 206, 381-386 (1995).

34. **Yee, J.K.** "Retroviral vectors and human gene therapy." **Mental Retardation & Development Disabilities Research Reviews** 1, 14-18 (1995).

35. Friedmann, T. and **Yee, J.K.** "Pseudotyped retroviral vectors for studies of human gene therapy" **Nature Medicine** 1, 275-277 (1995).

36. Burns, J.C., McNeill, L. Shimizu, C., Matsubara, T., **Yee, J.-K.,** Friedmann, T., Kurdi-Haidar, B., Maliwat, E., and Holt, C.E. "Retroviral gene transfer in Xenopus cell lines and embryos." **In Vitro Cell. Dev. Biol.** 32, 78-84 (1996).

37. Iida, A., Chen, S.T., Friedmann,T. & **Yee, J.K.** "Inducible gene expression by retrovirus-mediated transfer of a modified tetracycline-regulated system." **J. Virol.** 70, 6054-6059 (1996).

38. Chen, S.T., Iida, A., Guo, L., Friedmann, T. & **Yee, J.K.** "Generation of packaging cell lines for VSV-G pseudotyped retroviral vectors using a modified tetracycline-inducible system." **Proc. Natl. Acad. Sci. USA** 93, 10057-10062 (1996).

39. Yam, P.Y.Y., **Yee, J.K.,** Ito, J.I., Sniecinski, I., Doroshow, J.H., Forman, S.J. & Zaia, J.A. "Amphotropic and pseudotyped VSV-G retroviral transduction of human CD34+ peripheral blood progenitor cells (PBPC): Comparison in PBPC from adult donors with HIV-1 infection or with cancer." **Experimental Hematology** 26, 962-968 (1998).

40. Gasmi, M., Glynn, J., Jin, M.J., Jolly, D.J., **Yee, J.K.** & Chen, S.T. "Requirements for efficient production and infectivity of HIV-1 based vectors." **J. Virol**. 73, 1828-1834 (1999).

41. Johnston, J.C., Gasmi, M., **Yee, J.K.,** Jolly, D.J., Elder, J.H. & Sauter, S.L. "Minimal requirements for efficient transduction of dividing and nondividing cells by feline immunodeficiency virus vectors." **J. Virol.** 73,4991-5000 (1999)

42. Chow, W.A. , Fang, J.J. &**Yee, J.K.** "The IFN regulatory factor family participates in regulation of fas ligand gene expression in T cells." **J Immunol.** 164, 3512-3518 (2000)

43. Kowolik, C.M., Hu, J. & **Yee, J.K.** "The locus control region of the human CD2 gene in a lentiviral vector confers position-independent transgene expression" **J. Virol.** 75, 4641-4648 (2001).

44. Peng, H., Chen, S.-T., Wergedal, J.E., Polo, J.M., **Yee, J.K.,** Lau, K.-H.W. & Baylink, D.J. "Development of an MFG-based vector system for secretion of high levels of functionally active human BMP4" **Mol. Ther**. 4: 95-104 (2001).

45. Yam, P.Y.Y., Li, S., Wu, J., Hu, J., Zaia, J.A. & **Yee, J.K.** "Design of HIV vectors for efficient gene delivery into human hematopoietic cells" **Mol. Ther.** 5:479-484 (2002).

46. Kowolik, C.M. & **Yee, J.K.** "Efficient hepatocyte-specific transduction with lentiviral vectors" **Mol. Ther.** 5:762-769 (2002).

47. Kowolik, C.M., Yam, P., Yu, Y. & **Yee, J.K.** "HIV vector production mediated by Rev protein transduction" **Mol. Ther.** (in press).

Book Chapter

1. Rosenberg, M.B., Levine, F. and **Yee, J.-K.** "Genetics" in **Fetal and Neonatal Physiology**, Polin, R.A. and Fox, W.W., eds. W.B. Saunders Company, Philadelphia, pp 1-18, (1992).

2. **Yee, J.-K.** "Pseudotype-retroviral vectors" (Unit 12.8) in **Current Protocols in Human Genetics**, Dracopoli, N.C., Haines, J.L., Korf, B.R., Moir, D.T., Morton, C.C., Seidman, C.E., Seidman, J.G. and Smith, D.R., eds. John Wiley & Sons, Inc., (1997).

3. **Yee, J.-K.** "Retroviral vectors" in **The Development of Human Gene Therapy**, Friedmann, T., ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, Chapter 2, (1998).

4. **Yee, J.-K.** & Zaia, J.A. "Prospect for gene therapy using HIV-based vectors" in **Lentiviral Vectors for Gene Therapy**, Bushschacher, G., ed., Landes Bioscience, (2002)